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Cancer and Immunology Session

Dietary polyunsaturated fatty acids as cancer preventive tools in the modulation of lipid profiles associated with carcinogenesis

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Deregulation of cell growth and survival in cancer development has been associated with an altered fatty acid (FA) metabolism linked to the modulation of membrane structure and cellular oxidative status resulting in changes to membrane function, activity of enzymes and signal transduction pathways. A growing body of research supports the use of conjugated linoleic acid and n-3 PUFA in the prevention of certain cancers, especially regarding the imbalance in n-6 and n-3 polyunsaturated FA (PUFA) intake. In rat, a characteristic lipid profile is associated with the growth and development of preneoplastic lesions. This profile entails decreases in the PC/PE phospholipid ratio, C20:4n-6 PC/PE ratio, n-3 PUFA content and oxidative status. An interactive role of SATS, MUFA and C20:4n-6 are highlighted as a driving force sustaining altered growth characteristics in these lesions with a diet high in n-6 PUFA playing an underlying role. Lipid analyses of liver biopsies from human patients with hepatocellular carcinoma indicated a similar altered lipid pattern that is reduced n-6 PUFA level in PC, C20:4n-6 PC/PE ratio, n-3 PUFA in PC and PE phospholipids with a resultant decrease in oxidative status. These changes may serve as biomarkers for cancer development. In rat liver, this characteristic profile was modulated by dietary fat with varying low n-6/n-3 FA ratios (SFO/EPA, 12:1 ratio; SFO/EPA/GLA, 12:1 ratio; SOY, 5:1 ratio) resulting in an increased n-3 PUFA content, a higher lipid peroxidation level and altered growth of $GSTP^+$ preneoplastic lesions. It is suggested that modulation of cancer tissue lipid profile and oxidative status with dietary PUFA within a specific n-6/n-3 ratio may be an important chemopreventive tool in altering the growth characteristics of cancer cells. The association of CLA, other n-6 and n-3 FA with membrane dynamics in the regulation of cell growth and alteration in cellular oxidative status will be discussed.

Key words: Cancer, n-6 and n-3 polyunsaturated fatty acids, phosphatidylcholine, phosphatidylethanolamine.

Generating an oxidative stress model in human skin cells for antioxidant testing

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A low level of reactive oxygen species (ROS) is physiologically relevant in the functioning of normal cellular processes. However, high levels of ROS cause oxidative damage to cellular proteins, DNA and lipids. All of these may contribute to aging, progression of cardiovascular disease and cancer development. Cells counteract this damage by employing endogenous antioxidants and antioxidative enzymes that detoxify ROS. However, continued oxidative stress eventually overwhelms the cellular protection mechanism. Therefore, the addition of exogenous antioxidants could be helpful in the prevention of ROS-mediated damage. Novel antioxidant polymers produced through biocatalysis, have shown to have increased antioxidant activity and may have potential protective effects as exogenous compounds in human skin cells. The aim of this project was therefore to establish a model to test antioxidant efficacy in human skin cells. Keratinocytes and fibroblasts representatives of the epidermis and dermis, respectively, were exposed to different UVA doses (5, 10 and 20 J/cm^2). Cells were tested for the enzymatic conversion of tetrazolium salt XTT, to formazan as a measure of cell viability. ROS levels were measured using dihydrorhodamine 123 and the subsequent fluorescence was measured by flow cytometry. Purified DNA was visualised by agarose gel electrophoresis to assess DNA damage. The results showed that the 2 cell types respond differently to UV. In keratinocytes, the response was immediate in terms of mitochondrial activity, more ROS was produced, and DNA damage was observed 8 h after UV. In fibroblasts, the metabolic response to UV was slower, observed 24 h time point, and less ROS was produced. In conclusion, a model has been established for the assessment of the protective effects of antioxidants; whereby cell viability and DNA damage will be tested 4 and 8 h after UV irradiation in keratinocytes, while in fibroblasts cell viability will be evaluated after 24 h.

Key words: ROS, keratinocytes, fibroblasts, tetrazolium salt, formazan, dihydrorhodamine 123.

Depigmentation in melanomas increases the efficacy of hypericin-mediated photodynamic-induced cell death

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Melanoma is the main cause of death in skin cancers. Despite early detection, resection and post-operative therapy, combating melanoma remains ineffective and investigations into other forms of adjuvant therapy such as photodynamic therapy (PDT) are prudent. This study proposes that depigmentation that is the removal of the free radical scavenging pigment, melanin, in melanotic melanoma cells increases their susceptibility to PDT-induced cell death. Two human melanoma cell lines: one pigmented (Mel-1) and one amelanotic (A_{375}) cell lines were used. Kojic acid (KA), a tyrosinase-specific inhibitor, was shown to inhibit melanin synthesis after a 3-day exposure. Cells were then treated with Hypericin (HYP) PDT and cell viability measured. Intracellular reactive oxygen species (ROS) was also measured. Mode of cell death was checked by measuring the Caspase 3/7 activity just after 4 h of activation. PDT on KA treated cells resulted in a 3.82 fold increase of ROS production which correlated to 11% increase in cell death susceptibility compared to untreated controls. Moreover, cells allowed to regain their pigment which failed to return to normal even after 72 h thus proving the effectiveness of PDT. Using a DPPH* assay, the results confirmed the scavenging properties of melanin (IC5018.30 µg/ml) proving that this pigment may be one of the reasons for melanoma chemoresistance. There is no significant difference was observed in the caspase 3/7 activity in KA treated and none KA treated melanoma after the activation of 3 µM hypericin in comparison to the only UV treated control pigmented melanoma cell line. This

suggests that the HYP-PDT treatment induced a caspase independent cell death mechanism. Overall, this study shows that pigment plays an important role in the efficacy of adjunctive PDT treatment and its removal enhances cell death susceptibility in melanomas.

Key words: Melanoma, photodynamic therapy, kojic acid, hypericin, reactive oxygen species.

Evidence of cell diversity in the MCF10a human mammary cell line

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Human MCF10A cells represent apparently phenotypically normal luminal epithelial cells of the breast that form acini (rings of cells surrounding a cleared lumen) in three dimensional (3D) culture in the presence of basement membrane (BM) matrix components. While MCF10A cells intrinsically secrete BM components, only myoepithelial, and not luminal epithelial cells, have been described to secrete laminin-I, required for polarized acinus formation. This suggests that 3D matrix components may influence MCF10A acinus formation, or that the cell line contains a myoepithelial subpopulation in addition to the epithelial cell type, supporting the stem cell proliferation model described for the breast. We adapted the hanging drop 3D culture model to investigate the formation of MCF10A acini in the absence of an added matrix, and to explore progenitor-type cell diversity recently suggested for MCF10A cells. Hanging drop cultures were establish from small volumes of medium (30 µl drop), containing a low initial cell number (± 10^3 cells), pipetted onto the lid of a culture dish. After 10 days in culture spheroids containing acini and free-floating individual cells, were transferred to coverslips and fixed, stained for confocal microscopy or processed for SEM analysis. For TEM investigations, hanging drop cultures were embedded in low melting point agar to trap free-floating cells, fixed, dehydrated and embedded in resin. Confocal microscopy showed clear acinus-like structures, while SEM showed the spatial distribution of both acini and surrounding cells not proliferating into acini. TEM confirmed intercellular adhesions between acinar cells and normal ultrastructure of individual cells. Formation of polarised acini by MCF10A cells in hanging drop culture and the presence of non-participating cells suggests the presence of both myoepithelial and luminal epithelial lineages in this cell line. Application of this technique is underway in an invasive cancer cell model, in which both acinus formation and BM deposition is compromised.

Key words: MCF10A cells, laminin-I, acini, cancer, basement membrane.